United States Department of Agriculture
Grain Inspection, Packers and Stockyards Administration
Federal Grain Inspection Service

Program Notice FGIS-PN-14-01 01/16/14

REVEAL Q+ FOR AFLATOXIN GREEN TEST METHOD

1. PURPOSE

The purpose of this program notice is to provide instructions for the Neogen Reveal Q+ for Aflatoxin Green test method, product number 8086, which uses lateral flow assay technology and water-based extraction that eliminates the use of organic solvents (methanol, ethanol, etc.) to report aflatoxin results in parts per billion (ppb) in corn, and additional commodities (corn flour, corn grits, corn meal, flaking corn grits, and hominy). The Reveal Q+ for Aflatoxin Green test method has been approved by the Technology and Science Division (TSD) for official use.

2. BACKGROUND

The TSD provides performance evaluations of mycotoxin test kits for manufacturers seeking approval of their product for use in the official inspection system. The Policies, Procedures, and Market Analysis Branch (PPMAB) provides instructions for the use of approved mycotoxin test kits. In an effort to offer official use of the Reveal Q+ for Aflatoxin Green test method in a timelier manner, PPMAB has decided to issue this program notice prior to the release of the revised Mycotoxin Handbook. The following test procedures are approved for use by official testing locations.

3. TEST PROCEDURES

a. General Information.

The Reveal Q+ for Aflatoxin Green test method provided by the Neogen Corporation is a single-step lateral flow immunochromatographic assay based on a competitive immunoassay format. The test provides quantitative analysis for the presence of aflatoxins, using a non-hazardous, water-based extraction solution along with an aflatoxin-antibody particle complex coated test strip and the Neogen AccuScan Pro reader. Following approved test instructions and Neogen's assay principles the inspector can quantitatively determine the presence of aflatoxins in a sample.

The test kit is limited to providing quantitative aflatoxin measurements between 5 and 100 ppb. For reporting test results above 100 ppb, a supplemental (diluted sample) analysis must be performed or test results are reported as exceeding 100 ppb. Maximum supplemental analysis reporting limit is 200 ppb.

Obtain samples according to the instructions in the Grain Inspection Handbook, Book I "Grain Sampling," and refer to the Aflatoxin Handbook Chapter 3 "Sample Preparation for minimum sample requirement and sample preparation procedures."

Federal Grain Inspection Service (FGIS) employees must comply with all applicable safety and sanitation requirements as listed in this program notice to ensure a safe and efficient work environment. Refer to all Federal, (*Environmental Protection Agency, Title 40 CFR*), State, and local laws and regulations applicable to the management of hazardous waste.

<table>
<tr><th colspan="6">Approved Test Kit Information</th></tr>
<tr><td>Test Kit Vendor:</td><td colspan="5">Neogen Corporation 1-800-234-5333</td></tr>
<tr><td>Test Kit Name:</td><td colspan="5">Reveal Q+ for Green Aflatoxin Test Method</td></tr>
<tr><th>Product Number</th><th colspan="2">Conformance Limits</th><th>Type of Service</th><th>Temp Range</th><th>Supplemental Analysis</th></tr>
<tr><td>8086</td><td>Min</td><td>Max</td><td rowspan="3">Quantitative</td><td rowspan="3">64-86° F</td><td rowspan="3">Yes</td></tr>
<tr><td>FOL Code</td><td rowspan="2">5 ppb</td><td rowspan="2">100 ppb</td></tr>
<tr><td>AFLGD</td></tr>
<tr><td colspan="2">Grain/Commodities Approved for</td><td colspan="4">Corn, corn flour, corn grits, corn meal, flaking corn grits, and hominy.</td></tr>
<tr><td>Test Format:</td><td colspan="5">Single-step Lateral Flow Strip.</td></tr>
<tr><td>Extraction Method:</td><td colspan="5"><u>Blend only</u>: 50 gram sample with <u>250 mL</u> Green Extraction Solution (water-based) for 0.5 minutes.</td></tr>
<tr><td>Detection Technique:</td><td colspan="5">AccuScan Pro Reader only.</td></tr>
</table>

b. Preparation of Extraction Solution.

The extraction solvent used in the Reveal Q+ for Aflatoxin Green test method is a non-hazardous water-based extraction solution consisting of Neogen Green Extraction **Step 1**, **Step 2**, and 1 liter of distilled/deionized water.

(1) Prepare the extraction solution by adding 1 bottle of Green Extraction **Step 1** followed by 1 bottle of Green Extraction **Step 2** (*<u>Shake Green Extraction step 2 before adding to other solutions</u>*) into 1 liter of distilled/deionized water. Rinse the green extraction **Step 2** bottle twice with the extraction mixture to make sure everything (**Step 2** bottle contents) is removed and thoroughly mixed into the solution.

(2) Shake the extraction solution to mix. <u>Make sure all components are added together before mixing.</u>

(3) Label the container stating the mixture (Green Extraction Solution), date of preparation, and initials of the technician who prepared the solution.

The prepared Green Extraction solution is stable for 5 months when stored at ambient room temperature in a tightly closed container. One liter of Green Extraction solution is enough to run approximately 4 tests.

NOTE: Test kit includes enough Green Extraction components (Step 1 and Step 2) to analyze 8 test samples. Contact Neogen to order additional Green Extraction components as needed.

c. AccuScan Pro System Reader Set-up.

(1) Set-up:

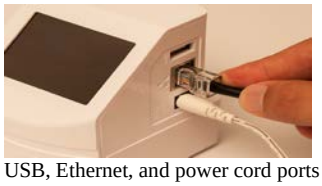
Press the Home icon at any time to return to the main screen.

(a) Plug the outlet of the cord into the power source. Plug the small end of the power cord into the reader.

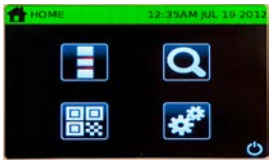
(b) This will turn the reader on. To turn the reader off, unplug the reader.

Note: To place in the power down mode, press the small power icon in the lower right corner of the Home screen. The screen will turn white and the reader may then be unplugged.

(c) Plug the USB key into the USB port on the side of the reader.


USB, Ethernet, and power cord ports

Start up screen



Home screen

(2) Settings:

Press the designated icon to set time, date, volume, remote connection, language setting, and allows the user to review current information.

(3) QR Codes:

The reader is capable of reading information from predefined QR codes. Disposable QR codes containing lot specific information is included in each Reveal Q+ kit.

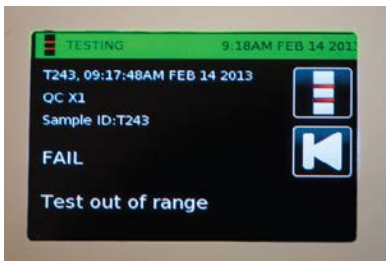
(a) Whenever a new lot is obtained and before you run a test you must verify the QR codes. Simply place the QR code card into the QR code cartridge. From the **Home screen**, select the QR code icon and place the QR code cartridge containing the card in the reader. The specific information automatically will be downloaded into the reader. The analyst needs to make sure that the information generated by the QR code on the reader matches the AccuScan Pro Curve Set shown on the Certificate of Analysis (COA). If the QR code information does not match the COA, then the analyst shouldn't use the test kit lot before consulting the manufacturer to resolve the discrepancy. Contact PPMAB and TSD to report this issue and the manufacture's response.

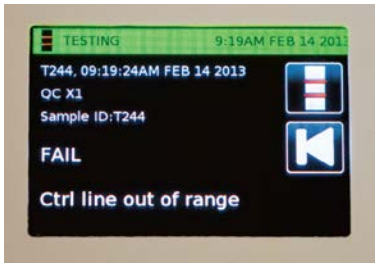
(4) Calibration/Normalization:

Calibration and Normalization settings can be found in the information/about section under the settings menu. From the Home screen select settings (gear icon) and then select the information/about icon. Use the arrow key to toggle back and forth through the menus.

(a) Test Line Calibration:

Test Line Calibration should be performed on a regular basis, at least every six months. This is considered field calibration and it will ensure the reader is interpreting the lateral flow devices correctly. Test Line Calibration uses the X1 strip provided with the system.





To perform the test line calibration, select **Run Test** from the Home Screen Icon and select the **Calibration** category. Select **QC X1** to perform the Test Line Calibration. Using the provided "A" cartridge, place the X1 test strip in the strip carrier. Make sure the lines on the test strip are within the white dots on the cartridge. Enter the carrier into the reader. The analysis will automatically begin and will provide one of three possible outcomes. The first outcome, "***PASS QC Ok***", indicates a passing result while the other outcomes, "***FAIL Ctrl out of range***" or "***FAIL Test out of range***", indicate a failed calibration. If the Test Line Calibration fails please call Neogen for further instruction. See above for reader display menu examples.

NOTE: If the test type package included with the reader does not include a category called "Calibration", contact Neogen to update the test type package.

(5) Run test protocol:

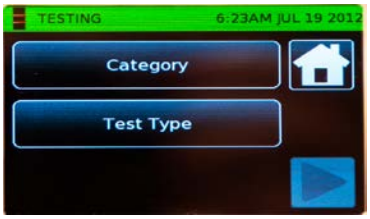
(a) Touch the **Run test** icon, which looks like a test strip, on the **Home screen**.

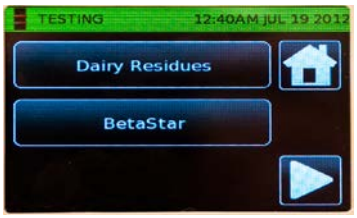
(b) The test category will display in the first box and the test type will display in the second box. The reader will default to the last test analyzed.

(c) Select the appropriate test category by touching the desired option.

(d) Touch the green check mark to confirm the selection.

(e) Touch the **Test type** icon. Select the desired test by touching the name of the test (e.g., BetaStar, Q+ Aflatoxin). To scroll through the test options, use the white up and down arrows. Touch the green check mark to confirm the selection.





(f) Insert the test strip to begin interpreting the test or enter the sample ID by touching the **Sample ID** button. **NOTE**: Strips may be tested without a sample ID. To do this, insert the test strip cartridge containing the test strip into the reader square end first. The reader automatically will begin analyzing the strip.

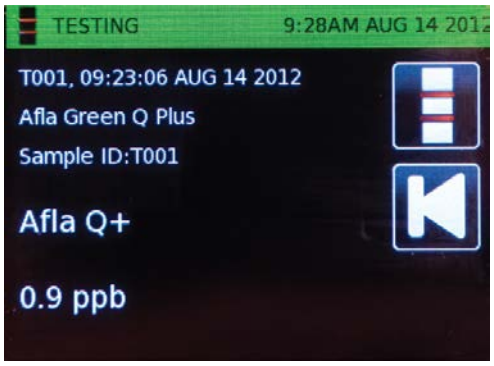
(g) If entering a sample ID, type the ID in and press the green check mark to continue.

(h) Insert the test strip into the appropriate strip cartridge. Insert the cartridge containing the strip into the slot on the lower left portion of the reader. The reader automatically will begin analyzing the strip.

(6) Test Results:

(a) For a standard positive/negative test, the screen will display results as a +POS for a positive sample and – NEG for a negative sample. For a quantitative test, a numerical value will be displayed.

(b) Touch the screen to view additional test information such as test line and control line ratios.



(c) To print, select the **Print** icon. A report will automatically be sent to the attached printer.

(d) To begin/run a new test, select the **New test** icon. This will return the user to the test setup screen.

<u>Reader Notes and Cautions</u>:

- Ensure device is fully inserted into the cartridge.
- Removing the cartridge prior to completion can result in invalid readings.
- Readings should be taken within 1 minute after the incubation is completed. The incubation time is 6 minutes. Any results taken after 7 minutes may be inaccurate due to over development of the strip and should not be reported.

- The strips must be read using only Neogen's Reveal AccuScan Pro reader only.

d. Extraction Procedures for approved grain and commodities.

(1) Transfer 50 grams (+/- 0.2 g) of ground sample into an extraction mixing jar.

(2) Add 250 milliliters (mL) of Green Extraction solution.

(3) Cover the extraction jar and blend for 30 seconds (0.5 minutes).

(4) Allow the sample to settle for 3 minutes. Then filter with Whatman No 1 filter paper using the reverse filtration method. Care should be taken to make sure that during the reverse filtration process the filter paper does not get immersed or is tilted into the filtering solution.

Note: The use of Whatman No. 1 filter paper is the only GIPSA-approved filtering procedure for this test kit. Do not use any filtering syringes or coffee filters for filtering.

(5) After enough filtrate is collected for testing, proceed to the test procedures section.

(6) Properly dispose excess sample filtrate and filter paper according to non-hazardous waste management guidelines when testing is complete.

e. Test Procedures.

(1) Sample Analysis.

(a) Place the appropriate number of red sample dilution cups and clear sample cups for each test sample in the sample cup rack. Label cups if necessary.

(b) Using a single-channel pipettor with a new pipette tip, add 500 microliters (µL) of sample diluent to each red sample dilution cup.

(c) Using a new pipette tip, add 100 µL of test sample extract into each red dilution cup with sample diluents. Mix by swirling with the pipette tip and then pipetting up and down 5 times.

(d) Transfer 100 µL of diluted sample extract into a new clear sample cup.

(e) Place a new Reveal Q+ for Aflatoxin Green test strip with the sample end down into the sample cup. Start timer and incubate for **6 minutes**.

(f) At the end of the 6 minute incubation/development period, remove the test strip from the sample cup. Read the test strip within 1 minute using the Neogen's Reveal AccuScan Pro Reader.

(2) Reading Test Results.

(a) The test strips must be immediately read using Neogen's AccuScan Pro Reader to analyze test strip. Test results will be displayed and stored in the reader.

(b) Reading should be made between **6 and 7 minutes**. Reading results after 7 minutes may be inaccurate due to over development and should not be reported.

(c) Fully insert the Reveal Q+ test strip into the black cartridge adapter with the sample end first and results facing out.

(d) Insert the cartridge with test strip side up into the AccuScan.

(e) The reader will automatically begin analyzing the cartridge.

CAUTION: Removing the cartridge before completion of the 6 minute incubation can result in invalid results.

(f) The AccuScan Pro reader will analyze the test strip. Test results will be displayed and stored in the reader.

(g) Proceed to Reporting and Certifying Test results section.

(3) Supplemental Analysis Procedures.

If quantitative results are above the test kit maximum conformance limits (e.g. 100 ppb), test results are reported as exceeding the limit (>100 ppb). To report aflatoxin results above the 100 ppb, the sample extract must be diluted so that a value between 5 to 100 ppb is obtained. See Dilution Procedure below.

This diluted sample result/value must report below 100 ppb and then multiplied by the appropriate dilution factor so that the actual concentration of the original sample is obtained. The maximum reportable limit for this test kit is **200 ppb**. Supplemental analysis results above 200 ppb are reported as "exceeding the 200 ppb limit (> 200ppb)".

For example: If the filtered extract is diluted 1:1 (v/v) using extractions solvent, the dilution factor is two **(2)**.

Dilution Procedures:

(a) Combine 100 µL filtered extract with 100 µL of Green Extraction solution.

(b) Mix by swirling or pipetting up and down 5 times. This is the Diluted Filtered Extract sample used for supplemental analysis.

(c) Refer to Test Procedures section for analysis.

(d) Read and record results on the work record, then multiply the analytical results obtained from the AccuScan reader by 2 to obtain the actual aflatoxin concentration of the original test sample (show all results on the work record).

Example:		
	AccuScan Pro reader supplemental analysis results:	90 ppb
	Multiplied by the dilution factor	x 2
	Sample results -**TOTAL:**	**180 ppb**

f. Reporting and Certifying Test Results.

(1) Report all results on the pan ticket and inspection log to the nearest whole ppb.

(2) Sample results over 100 ppb are reported as greater than 100 ppb (> 100 ppb) unless applicant requested supplemental analysis certification.

(3) Supplemental analysis results that are above 200 ppb are reported as exceeding the limit (> 200ppb).

(4) Refer to the Aflatoxin Handbook, Chapter 4, Certification, for more detailed certification statements and procedures.

g. Cleaning Labware.

Prepare a solution consisting of dishwashing liquid and water. Completely submerge labware, wash thoroughly, and then rinse with a copious amount of clean water before reusing.

h. Waste Disposal.

The remaining extract and disposable materials are considered non-regulated hazardous waste by current federal regulations, and suitable for normal/routine trash disposal. Please review State and local regulations which may be more stringent to ensure compliance.

i. Equipment and Supplies.

(1) Materials Supplied in Test Kits.

(a) 25 Reveal Q+ for Aflatoxin Green test strips.

(b) 25 red sample dilution cups.

(c) 25 clear sample cups.

(d) 1 bottle of sample diluent.

(e) 2 bottles of Green Extraction **Step 1**.

(f) 2 bottles of Green Extraction **Step 2**.

(2) Materials, Supplies and Equipment required but not Provided.

(a) Timer. (Neogen item #9426)

(b) 100 µL pipettor (or equivalent) with pipette tips.

(c) 500 µL pipettor (or equivalent) with pipette tips.

(d) Sample collection cups with lids. (Neogen item #9428)

(e) Reveal sample rack. (Neogen item #9475)

(f) Reveal AccuScan Pro Reader. (Neogen item #9565)

(g) Disposable polyethylene transfer pipettes.

(h) Distilled Water.

(i) Filter paper, Whatman No. 1.

(j) Dispensing pump or graduated cylinder. (Neogen item #9448, #9447)

(k) Pipette tips, 200, & 1000 µL.

(l) Sample grinder.

(m) Scale capable of weighing 5 – 50 grams.

(n) Bottle, 1 Liter. (Neogen item #9472)

(o) Green Extraction kit. (Neogen item # 8087)

j. Storage Conditions and Precautions.

(1) Storage Conditions.

(a) Store kit components at **room temperature** (18 - 30ºC, 64°- 86°F) when not in use to ensure full shelf life.

(b) Test strips should remain capped in the original tubes until ready for use to ensure optimal performance.

(c) Do not freeze test kit components.

(2) Precautions.

(a) Do not use test kit components beyond the expiration date.

(b) Test strip development times, other than those specified in Test Procedures section, may give inaccurate results.

(c) Treat all used liquids, including sample extract, and labware as if contaminated with aflatoxins, gloves and other protective apparel should be worn at all times.

(d) To avoid cross-contamination, use clean glassware for each sample and thoroughly wash all glassware between samples.

(e) Mix Green Extraction solution thoroughly before each use. Green extraction solution normally appears turbid (not clear).

4. FILING

Retain a copy of this program notice until the Mycotoxin handbook is revised to include the test procedures stated herein.

5. QUESTIONS

Direct any questions concerning this program notice to Carl Jackson, Policies, Procedures, and Market Analysis Branch(PPMAB), at (202) 720-8286, or email at carl.jackson@usda.gov or Patrick McCluskey, PPMAB, at (816) 659-8403, or email at patrick.j.mccluskey@usda.gov.

/s/Robert Lijewski

Robert Lijewski, Director
Field Management Division